AMENDMENT

It is respectfully requested that the application be amended without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows.

IN THE SPECIFICATION

Please replace the paragraphs beginning on page 10, line 16 (after "Brief Description of the Drawings") with the following rewritten paragraphs:

Figures 1A-F show CTLA-4 VLD-Specific Oligonucletides.

Figures 2A and 2B show the polynucleotide sequence of complete cDNA encoding human CTLA-4 (2A) and polypeptide sequence of the VLD of human CTLA-4 (2B).

Figure 3 shows the phage display of CTLA-4 VLD STMs as gene 3 fusions on the surface of phage or phagemid. CTLA-4 VLD STMs are depicted as black spheroids; gene 3 protein is depicted as white spheroids; FLAG polypeptide is depicted in grey; genes are marked in a similar colour code and are depicted in an oval phage(mid) vector.

Figure 4 shows a schematic representation of the somatostatin polypeptide. Somatostatin (somatotropin release-inhibiting factor SRIF) in a cyclic 14-amino acid polypeptide. The cyclic nature is provided by a disulphide linkage between the cysteine residues at positions 3 and 14. The four residues which constitute the tip of the loop (Phe-Trp-Lys-Thr) are implicated in binding to members of the somatostatin receptor family.

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Figure 5 shows the size exclusion HPLC profiles of affinity purified CTLA-4 VLD and CTLA-4-Som3 STM. Recombinant human CTLA-4 proteins were expressed in E. coli host TG1 from vector pGC, purified from periplasmic extracts by anti-FLAG affinity chromatography and subjected to size exclusion chromatography on a calibrated Superose 12 HR column. The elution profiles of purified CTLA-4 VLD and CTLA-4-Soms3 STM are overlayed in this graph. CTLA-4 VLD comprises tetramer (21.86 min), dimer (26.83) and monomer (29.35 min). CTLA-4-Som3 STM comprises dimer (26.34) and monomer (29.28). Traces represent absorbance at 214 nm and are given in arbitrary units.

Figure 6 shows a schematic diagram of CTLA-4 VLD loop replacements. The constructs are labeled A-I. Construct A (CTLA-4 VLD: S2) represents the wild-type CTLA-4 extracellular V-domain, spanning residues 1-115. Constructs B (CTLA-4-Som1; PP2) and C (CTLA-4-Som1-Cys120; PP5) both contain the 14 residue somatostain polypeptide in CDR1. PP5 also

carries a C-terminal extension containing Cys120. Construct D (CTLA-4-Som3; PP8) contains the 14 residue somatostatin polypeptide in place of CDR3. In construct E (CTLA-4-HA2:XX4), CDR2 has been replaced with a haemagglutinin tag. In construct F (CTLA-4-Som1-Som3: VV3), both CDR1 and CDR3 have been replaced with the somatostatin polypeptide. In construct G (CTLA-4-Som-HA2-Som3: ZZ3) CDR1 and CDR3 are replaced with the somatostatin polypeptide whilst CDR2 is replaced with haemagglutinin tag. In construct H (CTLA-4-anti-lys:2V8), all three CDR loop structures have been replaced with the CDR loops from a camel anti-lysozyme V_HH molecule. Construct I (CTLA-4-anti-mel: 3E4) represents CTLA-4 VLD in which all three CDRs have been replaced by the VH CDR loops from anti-melanoma antibody V86 (Cai And Garen, 1997). PelB, cleavable pectate lyase secretion sequence (22 aa); flag, dual flag tag (AAADYKDDDDKAADYKDDDDK).

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Figures 7A-I show HPLC profiles of purified recombinant human CTLA-4 STMs. Recombinant CTLA-4 VLDs were expressed in E. coli host TG1 from vector pGC, purified from periplasmic extracts by anti-FLAG affinity chromatography and subjected to size exclusion chromatography on a calibrated Superose 12 HR column. The elution profiles of the purified proteins are shown. 7A shows CTLA-4 DIMER (PP5); 7B shows CTLA-4R (S2); 7C shows CTLA-4-HA2 (XX4); 7D shows CTLA-4-Som3 (PP8); 7E shows CTLA-4-Som1 (PP2); 7F shows CTLA-4-Som1-Som3 (VV3); 7G shows CTLA-4-Som-HA2-Som3 (ZZ3); 7H shows CTLA-4-anti-lys (2V8); 7I shows CTLA-4-anti-mel (3E4).). Traces represent absorbance at 214 nm and are given in arbitrary units.

Figures 8A-E show a comparison by size exclusion FPLC analysis of affinity purified CTLA-4 constructs synthesised using bacterial expression vector pGC or pPOW. Recombinant human CTLA-4R or its loop variants were expressed in E. coli host TOP10F', purified from periplasmic extracts by anti-FLAG affinity chromatography and subjected to size exclusion chromatography on a calibrated Superose 12HR column. The elution profiles of proteins expressed from vector pGC are shown on the left, whilst proteins expressed from vector pPOW are shown on the right. 8A shows wild-type CTLA-4 VLD (S2); 8B shows CTLA-4-Som1(PP2); 8C shows CTLA-4-Som3(PP8); 8D shows CTLA-4-Anti-lys(2V8); 8E shows CTLA-4-Som1-HA2-Som3(ZZ3).

Figures 9A-E show protein stability analysis. Stability of monomer preparations of CTLA-4 VLD and loop variant constructs was analysed by size exclusion fplc chromatography

on a precalibrated superose 12 hr (Pharmacia) column following several cycles of freeze/thawing. Aliquots of each protein were tested immediately after peak purification and following two cycles of freeze/thawing. 9A shows CTLA-4 VLD (S2); 9B shows CTLA-4-Som1 (PP2); 9C shows CTLA-4-Som3 (PP8); 9D shows CTLA-4-anti-lys (2V8); 9E shows CTLA-4-Som-HA2-Som3 (ZZ3).

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Figures 10A-B show the lysozyme binding characteristics of CTLA-4-anti-lys construct 2V8. 10A shows ELISA analysis; 10B shows BIAcore analysis.

Figure 11 shows screening of CTLA-4 VLD phagemid library on immobilised Sh bleomycin.

Figures 12A-C show screening of CTLA-4 VLD libraries in solution. 12A shows an FD library (5 washes); 12B shows a phagemid library (2washes); and 12C shows a phagemid library (5 washes).